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| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
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| 10/038,717 | 01/08/2002 | Yuki Wakabayashi | NITT.0052 | 8912 |

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| EXAMINER | |
|---|---|
| FREDMAN, JEFFREY NORMAN | |
| ART UNIT | PAPER NUMBER |
| 1637 | |

DATE MAILED: 09/13/2004

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/038,717 | WAKABAYASHI ET AL. | |
| | Examiner | Art Unit | |
| | Jeffrey Fredman | 1637 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *18 June 2003*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-4 and 6-14* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-4 and 6-14* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☒ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449 or PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____.
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: _____.

## DETAILED ACTION

### *Status*

1. Claims 1-4 and 6-14 are pending.

Claims 1-4 and 6-14 are rejected.

Any rejection which is not reiterated in this action is hereby withdrawn as no longer applicable. This action is non-final because new rejections were added which were not necessitated by Applicant's amendment. However, the Nyren rejection is also maintained, rewritten and extended to all the applicable claims.

### *Claim Rejections - 35 USC § 112*

2. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

3. Claims 1-4 and 6-14 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

It is vague and indefinite what is meant by "pyrophosphates" in claim 1, line 2. "Pyrophosphates" are not capable of degrading pyrophosphoric acid. It is likely that this is a typo and that pyrophosphatase is meant, and this interpretation is applied in the prior art rejection below. Similar errors appear in claims 2-4 and 6-14. Correction is required.

It is vague and indefinite what is meant by "via degrading extended a strand produced in the extension reaction". This phrase is simply unclear, since the language makes no sense.

***Claim Rejections - 35 USC § 102***

4. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

5. Claims 1-4, 6-9, 11, 12 and 14 are rejected under 35 U.S.C. 102(b) as being anticipated by Nyren et al (WO 98/28440).

Nyren teaches a method of analysis of DNA sequence of claim 1 (see abstract), comprising the steps of:

(a) degrading, by pyrophosphatase, pyrophosphoric acid contained in a reagent used for extension reaction of a DNA primer hybridized to a target nucleic acid through a complementary binding, and/or degrading, by apyrase, adenosine s'-triphosphate contained in the reagent (see page 6, where Nyren teaches that DNA, which is generated by extending a DNA primer hybridized to a target nucleic acid, is treated with an immobilized nucleotide degrading enzyme, and Nyren teaches that apyrase is the preferred enzyme at page 4);

(b) removing or inactivating the pyrophosphates and/or the apyrase in the reagent after the degrading step (see page 6, where Nyren inactivates the enzymatically treated sample by removing the immobilized enzyme from the reaction mixture);

(c) conducting the extension reaction (see page 6, where Nyren teaches that the mixture may then be extended); and

(d) detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step (see page 6 and see page 7 for methods of detection using luciferase).

As a particular comment, Nyren teaches addition of a pyrophosphatase in reagent solutions to minimize PPi contamination (see page 19).

With regard to claim 2, Nyren teaches immobilization of the apyrase (see page 6).

With regard to claim 3, Nyren teaches detection of chemiluminescence where solutions of different nucleotides are present, since the nucleotides are added sequentially one by one, therefore when all four nucleotides are used (as in figure 3, for example), the method of claim 6 would involve adding a pyrophosphatase to a sequence with each different nucleotide.

With regard to claim 4, Nyren teaches the conversion using adenosine 5' phosphosulfate and ATP sulfurylase (see pages 7 and 8) as well as the detection using chemiluminescence using ATP (see page 8).

With regard to claim 6, Nyren teaches adding pyrophosphatase to the solution iwth the DNA polymerase (see page 6, where the enzyme is added to the extension reaction which comprises at least a DNA polymerase, as well as ll the other listed components as discussed at pages 7 and 8).

With regard to claim 7, Nyren teaches removing the pyrophosphatase/apyrase by (see page 6).

With regard to claim 8, Nyren teaches the apyrase is immobilized on a solid (see page 6).

With regard to claim 9, Nyren teaches detection of SNPs (see page 1, last sentence to page 2, first sentence and pages 27-28).

With regard to claims 11-12, Nyren teaches addition of pyrophosphatase to each extension step (see page 6), which will include addition to each of the four dNTPs used in the sequencing reaction since the reaction sequentially uses all four dNTPs (see figure 3). The remaining steps are discussed above.

With regard to claim 14, Nyren teaches the use of polymerases, some of which have exonuclease activity (see page 16).

6. Claims 1, 3, 4, 6, 7 and 9 are rejected under 35 U.S.C. 102(b) as being anticipated by Nordstrom et al (Anal. Biochem. (2000) 282:186-193).

Nordstrom teaches a method of analysis of DNA sequence of claim 1 (see abstract), comprising the steps of:

(a) degrading, by pyrophosphatase, pyrophosphoric acid contained in a reagent used for extension reaction of a DNA primer hybridized to a target nucleic acid through a complementary binding, and/or degrading, by apyrase, adenosine s'-triphosphate contained in the reagent (see figure 1, panel a and page 187, subheading "Enzymatic preparation of dsDNA templates", where Nordstrom teaches that PCR amplified DNA, which is generated by extending a DNA primer hybridized to a target nucleic acid, is treated with pyrophosphatase and apyrase);

(b) removing or inactivating the pyrophosphates and/or the apyrase in the reagent after the degrading step (see figure 1, panel a and page 187, subheading "Enzymatic preparation of dsDNA templates", where Nordstrom inactivates the enzymatically treated sample by heating to 100 C);

(c) conducting the extension reaction (see figure 1, panel a and page 187, column 2, subheading "Pyrosequencing"); and

(d) detecting pyrophosphoric acid generated by the extension reaction after the removing or inactivating step (see figure 1, panel a and page 187, column 2, subheading "Pyrosequencing", where apyrase was used to detect pyrophosphoric acid in concert with luciferase).

With regard to claim 3, Nordstrom teaches detection of chemiluminescence (see figure 3, for example).

With regard to claim 4, Nordstrom teaches the conversion using adenosine 5' phosphosulfate and ATP sulfurylase (see page 187, subheading "Pyrosequencing" as well as the detection using chemiluminescence using ATP (see page 187, subheading "Pyrosequencing").

With regard to claim 6, Nordstrom teaches adding pyrophosphatase to PCR product prior to sequencing (see page 187, subheading "enzymatic preparation of dsDNA templates) which product includes DNA primer and DNA polymerase.

With regard to claim 7, Nordstrom teaches inactivating the pyrophosphatase and apyrase by heating to 100 C (see page 187, subheading "enzymatic preparation of dsDNA templates).

With regard to claim 9, Nordstrom teaches sequencing the 16S rRNA gene (see figure 3) which sequence inherently comprises SNPs.

***Claim Rejections - 35 USC § 103***

7. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

8. This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

9. Claims 10 and 13 are rejected under 35 U.S.C. 103(a) as being unpatentable over Nyren et al (WO 98/28440) in view of Ishikawa et al (Human Immunology (1995) 42:315-318).

Nyren teaches a method of claims 1-9, 11, 12 and 14 as discussed above. Nyren does not teach the use of mismatched primers.

Ishikawa teaches that putting mismatches in primers near the 3' termini increases the specificity of amplification (abstract and page 316, column 2).

It would have been prima facie obvious to one of ordinary skill in the art at the time the invention was made to modify the sequencing method of Nyren to use primers which have been modified to improve specificity as taught by Ishikawa since Ishikawa states "the introduction of an additional one-base mismatch is a simple and useful way to improve the specificity (page 316, column 2)". An ordinary practitioner would have been motivated to modify the primers of Nyren by creating mismatches near the 3' end in order to improve the specificity of the single base extension reaction, thereby improving the quality of the assay and reducing the number of false negative and false positives which would otherwise occur, thereby increasing the specificity of the sequencing reaction.

***Response to Arguments***

10. Applicant's arguments filed June 18, 2003 have been fully considered but they are not persuasive.

Applicant argues that Nyren does not teach removing the apyrase from the reagent solution after a degrading step. This is simply incorrect. As Nyren states "For example such immobilised enzyme (s) may be added after nucleotide incorporation (i.e. chain extension) has taken place, and then, when the incorporated nucleotides are hydrolysed, the immobilised enzyme may be removed from the reaction mixture (e.g. it may be withdrawn or captured, e.g. magnetically in the case of magnetic beads), before the next nucleotide is added. The procedure may then be repeated to sequence more bases. (see page 6)." This is an express teaching to remove the enzyme after the degrading step.

***Conclusion***

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jeffrey Fredman whose telephone number is (571)272-0742. The examiner can normally be reached on 6:30-4:00.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571)272-0782. The fax phone number for the organization where this application or proceeding is assigned is 703-872-9306.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

Jeffrey Fredman
Primary Examiner
Art Unit 1637
9/10/04